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TAXONOMIC IDENTITY OF *PORTUNUS PELAGICUS* SPECIES COMPLEX IN INDIAN WATERS USING MORPHOLOGICAL AND MITOCHONDRIAL DNA MARKERS

Jose Josileen*, A.Gopalakrishnan, P.R.Divya, V.S.Basheer and G.Maheswarudu

drjoslin@gmail.com

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Introduction

Portunus pelagicus (Linnaeus 1758), commonly known as the blue swimmer crab, is the most valuable marine crab landed in India. Though the species is distributed throughout the Indian coast, regular fishery exists in Palk Bay and Gulf of Mannar along the South East coast of India. Till recently, it was believed that *Portunus pelagicus* (Linnaeus, 1758) was the only species found in India, though colour variations were noticed in the male and females while studying the species for capture and culture works. Lai et al., (2010), has revised the species and four distinct species were recognised based on morphological and DNA characters as well as biogeographical considerations. Hence considering the urgency, the present work has initiated to rule out the taxonomic ambiguity existing among the species complex of *P. pelagicus* existing in Indian waters.

Materials and Methods

Specimen collection: Samples of *Portunus species* were collected from Palk Bay & Gulf of Mannar along the SE Coast of India. A portion of muscle tissue was collected and stored in 95% ethyl alcohol and stored at 4°C in a refrigerator.

Glimpses from sample collections

Isolation of DNA: **DNA extraction for all samples followed using Qiagen kit Blood and Tissue Kit (Cat No. 69506).**

Amplification of 16S rRNA &Cytochrome Oxidase I (COI) region:

The mitochondrial 16S rRNA and CO1 gene was amplified using following primers.

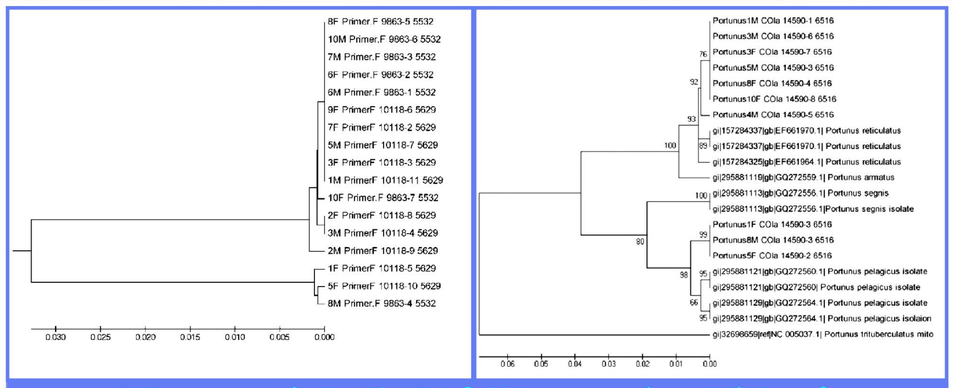
Genes	Primer Sequence
16S rRNA gene	H3080 16SAR (5'-CGCCTGTTTATCAAAAACAT-3') L2510 16SBR (5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi et al., 1991)
COI gene	Primer I. LCO 1490 5'-GGTCAACAAATCATAAAGATATTG-3') HCO 2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA 3') [Folmer et al., 2005] Primer II. COI (5'AGTATAAGCGTCTGGGTAGTC 3') COI (5' CCTGCAGGAGGAGGAGATCC 3') [Kessing et al. 1989]

Sequence analysis: Products were sequenced bidirectionally using ABI 3730 capillary sequencer. The raw DNA sequences were edited and aligned using BioEdit sequence alignment editor version 7.0.5.2. The Sequence divergence values within and between species were calculated using Kimura 2 Parameter (K2P) distance model implemented in MEGA V.5.0, Molecular Evolutionary Genetic Analysis software.

Results

Genetic analysis based on 16S rRNA gene and COI gene

16S rRNA gene	COI gene
450 base pairs of aligned sequences	655 base pairs of aligned sequences
Two clusters in the UPGMA tree with a divergence value 2.4-3.8 %.	Two clusters in the UPGMA tree species with a divergence value was 6.4-6.7 %.
Minor cluster (including 1F, 5F, 8M alone) represented *Portunus pelagicus*	Minor cluster (including 1F, 5F, 8M alone) represented *Portunus pelagicus*
Major cluster includes *Portunus reticulatus* (all individuals except 1F, 5F, 8M).	Major cluster includes *Portunus reticulatus* (all individuals except 1F, 5F, 8M).
Intra species divergence: *P. reticulatus*: 0.00-0.001/ *P. pelagicus*: 0.002-0.007	Intra species divergence: *P.reticulatus*:0.00-0.001/ *P.pelagicus*: 0.002-0.007
Haplotype diversity: 0.9123 Nucleotide diversity : 0.00815	Haplotype diversity: 0.9590 Nucleotide diversity : 0.04360



NJ Tree generated using 16s rRNA & COI sequences show similarity of cluster I with *Portunus reticulatus* and cluster II with *Portunus pelagicus*.

	1F	1M	2F	2M	3F	3M	5F	5M	7F	9F	6M	6F	7M	8M	8F	10M	10F
1F	0.000																
1M	0.066	0.000															
2F	0.067	0.002	0.000														
2M	0.069	0.003	0.005	0.000													
3F	0.066	0.000	0.002	0.003	0.000												
3M	0.067	0.002	0.000	0.005	0.002	0.000											
5F	0.002	0.064	0.066	0.067	0.064	0.066	0.000										
5M	0.066	0.000	0.002	0.003	0.000	0.002	0.064	0.000									
7F	0.066	0.000	0.002	0.003	0.000	0.002	0.064	0.000	0.000								
9F	0.066	0.000	0.002	0.003	0.000	0.002	0.064	0.000	0.000	0.000							
6M	0.066	0.000	0.002	0.003	0.000	0.002	0.064	0.000	0.000	0.000	0.000						
6F	0.066	0.000	0.002	0.003	0.000	0.002	0.064	0.000	0.000	0.000	0.000	0.000					
7M	0.066	0.000	0.002	0.003	0.000	0.002	0.064	0.000	0.000	0.000	0.000	0.000	0.000				
8M	0.003	0.066	0.067	0.069	0.066	0.067	0.002	0.066	0.066	0.066	0.066	0.066	0.066	0.000			
8F	0.066	0.000	0.002	0.003	0.000	0.002	0.064	0.000	0.000	0.000	0.000	0.000	0.000	0.066	0.000		
10M	0.066	0.000	0.002	0.003	0.000	0.002	0.064	0.000	0.000	0.000	0.000	0.000	0.000	0.064	0.002	0.000	
10F	0.064	0.002	0.003	0.005	0.002	0.003	0.062	0.002	0.002	0.002	0.002	0.002	0.002	0.060	0.001	0.002	0.000

Pair-wise genetic distances (Kimura 2-parameter) of Portunus species based on COI using MEGA V 5.0.

Summary & Conclusion

Data generated using COI and 16S rRNA sequences show two distinct clusters among *Portunus pelagicus* species distributed along Gulf of Mannar and Palk Bay- cluster I of *P.reticulatus* and cluster II *P. pelagicus*. The work is continuing with samples procured from the same area as well as from other naturally ocurring areas of the species, to get clear picture of *Portunus pelagicus* species diversity in the country.

Portunus pelagicus *Portunus reticulatus*

References

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3, 294-299.

Kessing et al. 1989 Kessing, B., H. Croom, A. Martin, C. McIntosh, W. O. McMillan & S. P. Palumbi, 1989. The Simple Fool's Guide to PCR Verson 1.0. Special Publication of the Department of Zoology, University of Hawaii, Honolulu. 45 pp.

Palumbi SR, Martin A, Romano S, Mcmillan WO, Stice L and Grabowski G (1991) The Simple Fool's Guide to PCR. University of Hawaii Press Honolulu.

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Central Marine Fisheries Research Institute, Cochin, INDIA-682 018

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